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ADDITIONAL EXPERIMENTS ON THE BEHAVIOR OF BUDS IN THE ASCIDIAN, *APLIDIUM MULTIPLICATUM*

MITSUAKI NAKAUCHI AND KAZUO KAWAMURA

Department of Biology, Faculty of Science, Kochi University, Asakura, Kochi 780, Japan

In the colonial ascidian, *Aplidium multiplicatum*, the strobilae produced in the abdomen and postabdomen migrate through the tunic and approach the regenerating thorax (their mother zooid) to form a common cloacal system with it (Nakauchi, 1966a, Nakauchi and Kawamura, 1974a). In a previous paper (Nakauchi and Kawamura, 1974b), a series of experiments were undertaken by the authors to study the mechanism by which the buds move in the "right" direction, and by which the buds and mother zooid form a system.

Three kinds of experiments were described in the previous paper: first, destroying the mother zooid; secondly, pulling out the mother zooid; and thirdly, pulling out the mother zooid together with the tunic covering it. The results of these experiments suggested the possibility that a substance secreted by each mother zooid diffuses through the tunic and attracts the growing buds.

In order to confirm the existence of the attractant and to determine the time and site of its secretion, four additional experiments were designed.

Material and Methods

A colonial ascidian, *Aplidium multiplicatum*, was used (see Nakauchi and Kawamura, 1974a). The experiments were done at the Usa Marine Biological Station of Kochi University, from March to June, 1974, at a sea water temperature of 18–22° C. For details of culture method and treatment of colonies prior to operations, see Nakauchi and Kawamura (1974b). The four experiments described in this report are numbered consecutively with those of the previous paper (Nakauchi and Kawamura, 1974b; Experiments I, II and III).

Experiment IV

The results of Experiment II and III suggested that the attractant is secreted from a budding (mother) zooid, and it remains in the tunic for a while even after the removal of the zooid. Experiment IV was designed to determine whether the substance is secreted only by budding zooids or whether it is also produced by nonbudding zooids. It is known in polycitorines (Oka and Watanabe, 1961) and in polyclinids (Freeman, 1971) that the removal of the thorax of a zooid is followed by strobilation of the abdominal region within one or two days. So, in this experiment the thorax of a grown zooid (a prospective mother zooid) was cut off, and the behavior of the experimentally-produced buds, which lack a mother zooid from the first, was followed. If the substance is secreted into the tunic even in the nonbudding period, the produced buds would aggregate near the place where

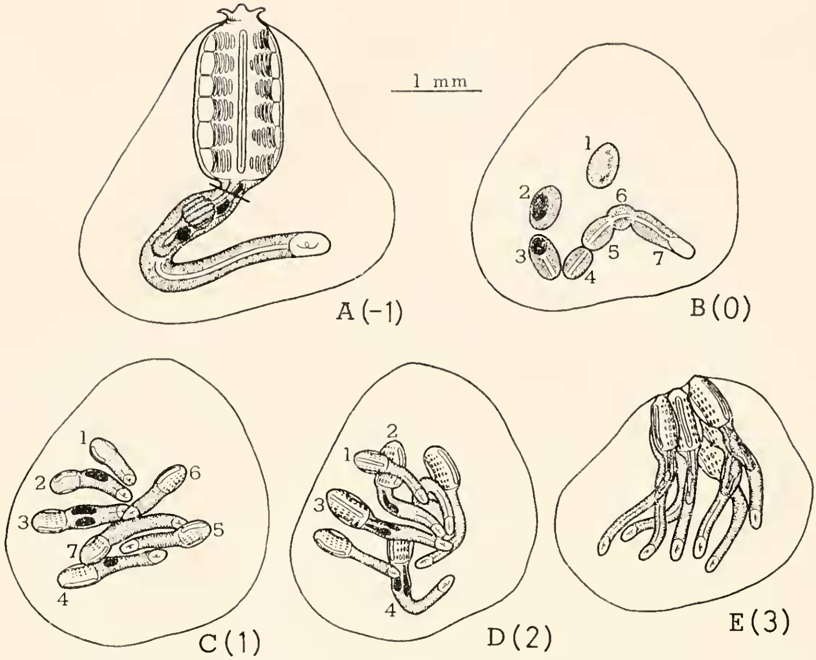

FIGURE 1. Behavior of buds in Experiment IV. Successive stages (A–E) in a typical case are viewed from the ventral side. The small arabic numbers identify individual buds in order from anterior to posterior, and the figures in parentheses indicate the number of days before or after budding.

the thorax had been located; if it is not, the grown buds should not aggregate at any definite place. They might aggregate at various places by chance, or open to the exterior independently without grouping. A total of 18 operations of this type were made.

Experiment V

In Experiment IV the growing buds arranged themselves near the place where the prospective mother zooid had been located. Therefore, in Experiment V the thorax of a grown zooid was cut off, together with the tunic surrounding it (Fig. 3A). By this procedure it was hoped to eliminate the attracting influence of the prospective mother zooid. A total of eight operations of this type were made.

Experiment VI

In normal budding, growing buds arrange themselves around the atrial aperture of mother zooid. It is plausible, therefore, that the attractant is most actively secreted by the epidermis around the aperture. So, in Experiment VI the anterior tip of the mother zooid was removed within one day after strobilation. At the same time, all the buds but one were also removed, and the behavior of the remaining bud was followed. A total of nine operations of this type were made.

Experiment VII

The result of Experiment IV suggested that the attractant is secreted not only by budding zooids but also by nonbudding zooids. Consequently, Experiment VII was designed to find out whether a grown (nonbudding) zooid has the potency to attract buds produced by other zooids in the same colony. For the convenience of observation, all the zooids but two were removed from the colony. After the operation the two remaining zooids came together, and a small colony consisting of only two zooids was formed. It was known that budding in a colony does not occur synchronously. In the present case, therefore, one zooid was expected to make buds earlier than the other, and we could hope to study the attractive influence of a nonbudding zooid to buds produced by the other.

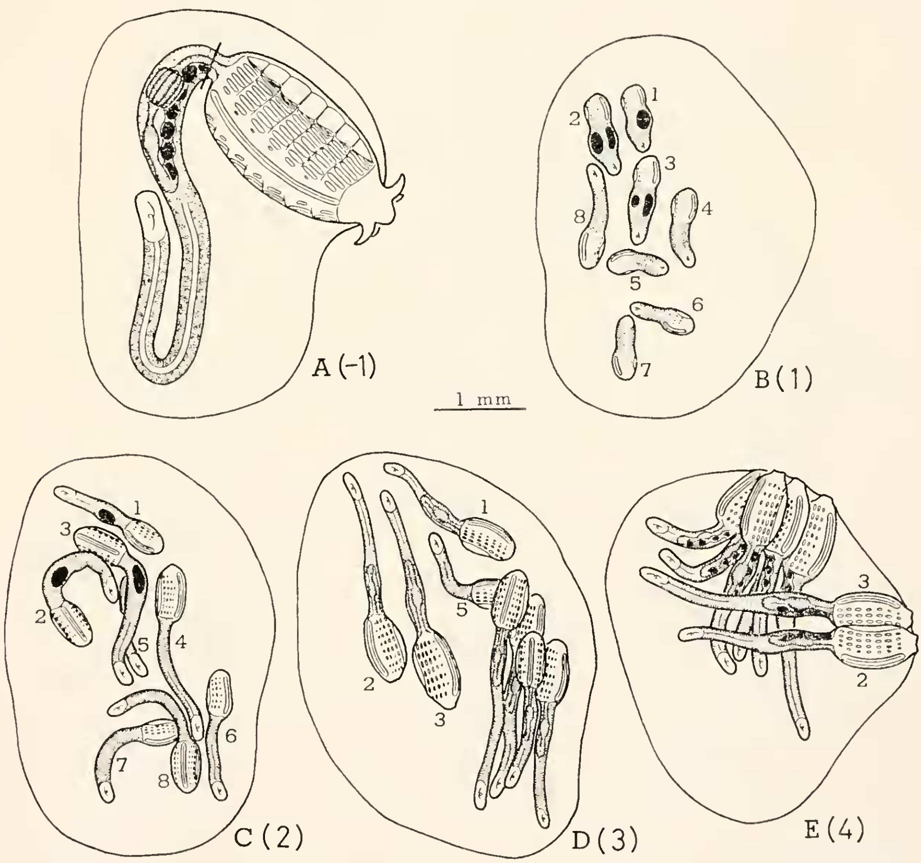

FIGURE 2. Behavior of buds in Experiment IV. Successive stages (A–E) in one of the minor cases are viewed from the ventral side. Bud identification numbers and days before or after budding are indicated as in Figure 1.

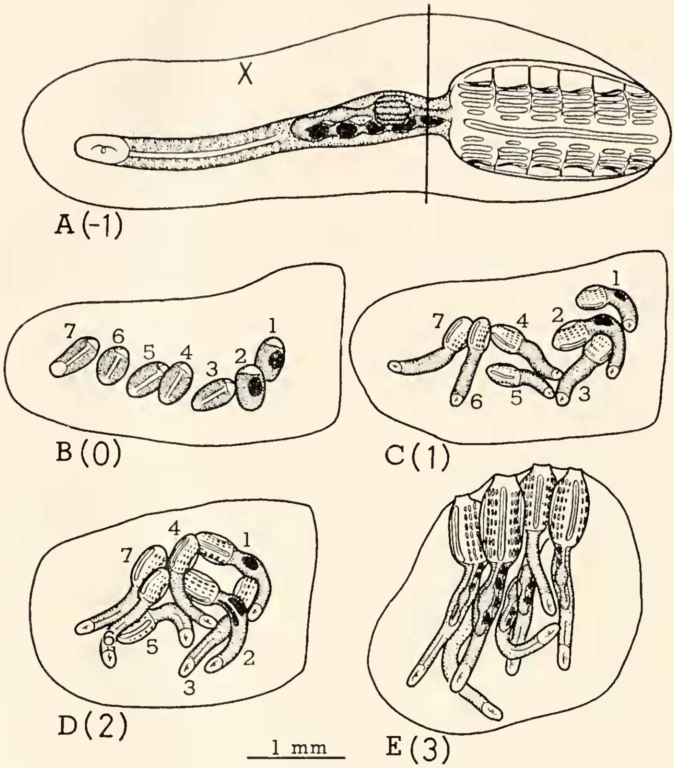

FIGURE 3. Behavior of buds in Experiment V. Successive stages (A–E) in a typical case are viewed from the ventral side. Bud identification numbers and days before or after budding are indicated as in Figure 1.

RESULTS

Experiment IV

In 14 cases out of 18, a single common cloacal system was formed by grown buds more or less near the place where the thorax (prospective mother zooid) had been located (Fig. 1). In the remaining four cases growing buds formed two groups and finally made two systems (Fig. 2). Even in the cases in which only one system was formed, the behavior of buds was somewhat different from that in usual budding. Buds lacking their mother moved more irregularly than in usual budding for about two days after budding (Fig. 1C–D). As a rule, the buds which had been originally located apart from the removed thorax needed more time to find the right direction than those located near the thorax. Following this stage the growing buds grouped to form a common cloacal system.

Experiment V

In five out of eight cases observed, one common cloacal system was formed by new zooid, while in the remaining three cases two systems were formed. Irrespective of the number of systems formed, the site of the common cloacal aperture did not appear to be influenced by the location of the prospective mother zooid which had been removed with its tunic before budding. That is, the systems did not

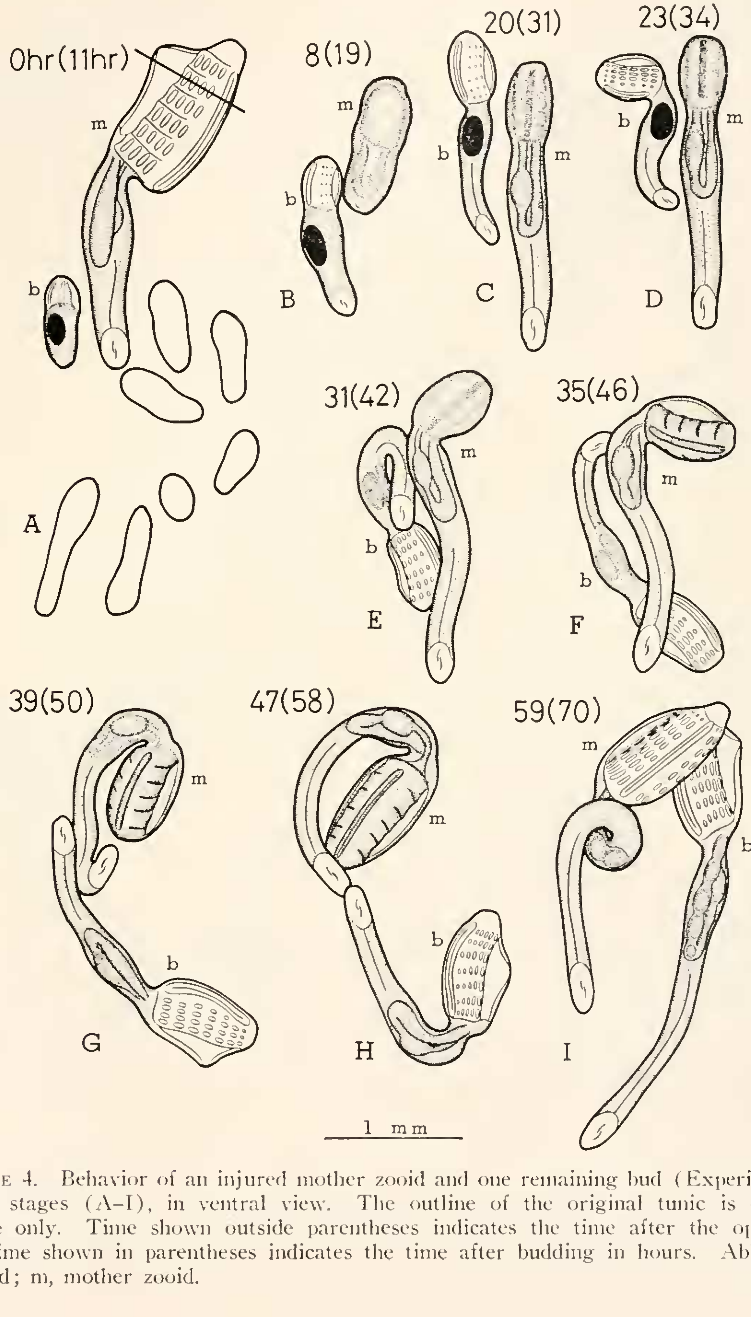

FIGURE 4. Behavior of an injured mother zooid and one remaining bud (Experiment VI), successive stages (A–I), in ventral view. The outline of the original tunic is omitted in this figure only. Time shown outside parentheses indicates the time after the operation in hours. Time shown in parentheses indicates the time after budding in hours. Abbreviations are: b, bud; m, mother zooid.

arise close to the cut surface of the tunic (Fig. 3). In most cases in which one system was formed, the new common cloacal aperture formed lateral to the position of the middle of the mother zooid's abdomen before strobilation (point "X" on Fig. 3A).

Experiment VI

In all nine cases the behavior of the single remaining bud looked strange. In eight cases the bud first moved forward, then turned and approached the abdomen or postabdomen of the mother zooid. This was followed by a complicated behavior of both bud and mother, the behavior of one apparently affecting the behavior of the other. After this, mother and bud arranged themselves side by side and finally made a common cloacal system. In the remaining exceptional case, the bud moved away from its mother zooid, and each opened to the exterior independently.

Figure 4 shows one of the major cases. The mother zooid contracted strongly after the operation; its thorax remained contracted for about 20 hours, while its new heart began to beat faintly about 8 hours after the operation. The bud moved toward the mother's thorax during the first 20 hours, then began to turn and finally pointed in the opposite direction (Fig. 4E). After this the bud moved toward the posterior end of the mother. On the other hand the mother, which had been turning very slowly, began a complicated behavior as the bud came near. Mother and bud changed their position as if they were affected by each other (Fig. 4, F–H), and they finally arranged themselves side by side (Fig. 4I) and made a system.

Experiment VII

Figure 5A shows two zooids left in the tunic, in which Zooid A is making ten buds. It was desired to eliminate the attracting influence of the thorax of Zooid A in order to see the attractive effect of Zooid B upon the buds produced by Zooid A. Thus, the thoracic bud of Zooid A was extirpated from the tunic. Four buds (3, 4, 9, and 10) were also cut off for the convenience of the observation. After these operations, Bud 1 and Bud 2 gradually moved toward the place where their mother had been located, and then they began to form a new system by themselves (Fig. 5B–D). On the other hand, Buds 5, 6, 7, and 8 approached the thorax of Zooid B (Fig. 5B); however, Zooid B made nine buds two days after Zooid A had budded. Of the nine buds of Zooid B, five buds (*1, 2, 3, 5,* and *6*) were incorporated into the system which was being formed by Buds 5, 6, 7, and 8 of Zooid A. The remaining four buds were, however, attracted by Buds 1 and 2 of Zooid A, and finally formed a system with them. As shown in Figure 5E, two systems were formed in a colony, each of which consisted of zooids of two different origins.

Discussion

The results of Experiments IV and V are consistent with the hypothesis that a substance secreted from the thoracic region of the mother diffuses through the tunic and attracts buds. It is plausible that in Experiment IV the substance

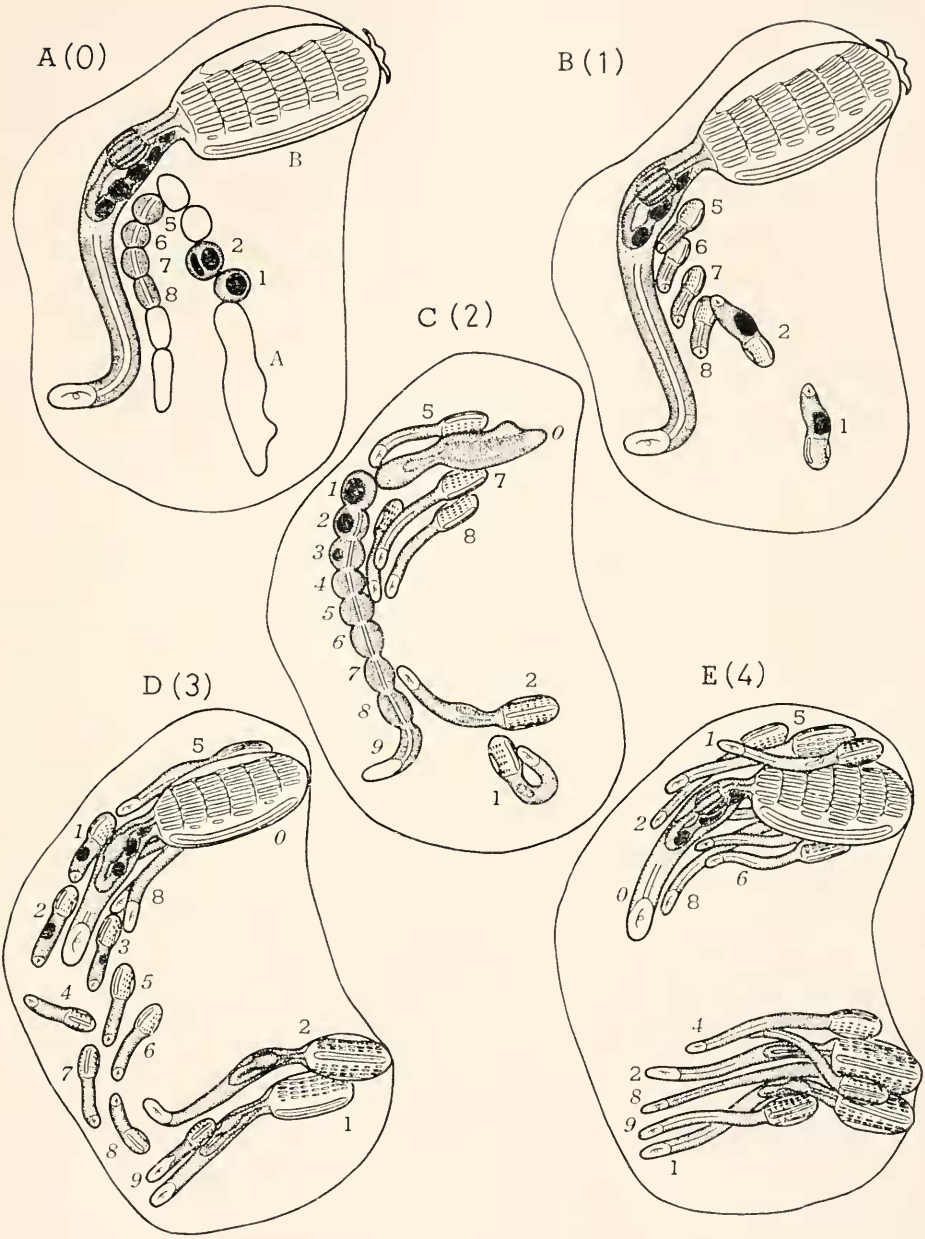

FIGURE 5. Behavior of buds in Experiment VII, successive stages (A–E), in ventral view. Time shown in parentheses indicates the time in days after budding of zooid A. The buds of Zooid A are identified by upright arabic numbers, and those of Zooid B by sloping ("italic") numbers.

remaining in the tunic after removal of the mother's thorax attracted the buds. This suggests that the period of secretion is not restricted to the time of budding. It is also likely that in Experiment V the removal of the adjacent tunic, as well as the maternal thorax, caused the elimination of the substance, and the buds became free of the influence of the mother. On the other hand, the buds appear to have influenced each other, seeing that they formed a system independent of the position of the "prospective mother zooid".

The result of Experiment VI is susceptible to various interpretations. The behavior of buds which have temporarily moved from the thoracic region of the injured mother may be explained at least in two ways. One is that the buds "dislike" the substance secreted by the wounded thorax, and they moved away from the vicinity of the wound. Another possibility is that the attractant is constantly secreted all over the body surface, although secretory activity shows an anteroposterior gradient with its high point at the most anterior region. When the anterior end is removed, the injury is accompanied by a reduction in secretory activity of the region, and the density of the attractant in the posterior region may become higher than that at the anterior. This temporary reversal of the polarity may last only until regeneration of the lost anterior end is completed. If this supposition is the case, one would expect the bud to move posteriorly at first, and then, after the recovery of the original polarity, to move anteriorly. Judging from the behavior of buds in other experiments, the latter case seems probable.

In Experiment VII it was shown that a bud is attracted not only by its mother but also by another grown (nonbudding) zooid or by a developing bud of another zooid. Because all the zooids in a colony originate from an oozooid, every zooid has the same set of genes. Consequently, zooids in a colony appear to have many characters in common. In other words, they lack individuality in many features. Therefore, an attractant secreted by one zooid may naturally attract all the buds in a colony regardless of their origin. The behavior of Buds 1 and 2 of Zooid A is understandable if we presuppose an attractant which had been secreted by the thorax of Zooid A and had diffused into the tunic before the thorax was removed. This result coincides with that of Experiment III shown previously (Nakauchi and Kawamura, 1974b).

Experiments are being undertaken to get more direct evidence of the existence of the attracting substance. The authors do not necessarily postulate a substance which has evolved specifically for the purpose of attraction. On the contrary, it is likely that growing buds are attracted by some metabolite produced by their mother zooid.

Setting aside the possible existence of an attracting substance, the movement of developing buds is known in many colonial ascidians. In polyclinids, most of which form common cloacal systems, Brien (1936) seems to have been the first to describe the movement of buds and its relation to system formation. Movement of buds is known even in colonial ascidians which form no systems (*Polycitor mutabilis*, Oka and Usui, 1944; *Metandrocarpa taylori*, Abbott, 1953, and Newberry, 1965; *Archidistoma aggregatum*, Nakauchi, 1966b; *Symplegma reptans*, Sugimoto and Nakauchi 1974; *Ritterella pulchra*, Nakauchi, 1977). Among these forms the buds of *Ritterella pulchra* can turn as much as 180°. We suggest that buds of colonial ascidians have some ability to move through the tunic and to change direction of movement. Some colonial ascidians may have exploited this ability for the purpose of system formation. In *Metandrocarpa* it is known that the common vascular system in the tunic plays an important role in the movement of buds (Newberry, 1965). Nothing is known of the mechanism of bud movement in polyclinid ascidians which have no common vascular system. However, buds and developed zooids are sometimes observed to contract and expand, and it is likely that this action is involved in the movement of zooids. The fact that all the buds and developed zooids can move forward only (Nakauchi and Kawamura, 1974a) may be a clue for solving this problem.

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Summary

In a previous paper by the authors, it was suggested that the behavior of growing buds, which form a common cloacal system, is affected by a substance which is secreted by the mother zooid and diffuses through the tunic.

Four sets of experiments were made to confirm the existence of the substance, and to get more information about the attractant. In the first set, the thorax of a grown zooid was removed before budding, and artificial strobilation was induced. In this case the buds lacked the mother zooid from the first. In the second, the thorax of a grown zooid was removed before budding, together with the tunic covering the thorax. In the third, the anterior tip of a mother zooid, thought to be a center of secretion, was removed. In the fourth, the experiment was designed to show whether a bud is attracted only by its mother and sisters or also by other zooids in the same colony. After these operations the behavior of buds was followed.

The results supported the existence of the attractant. They suggested that the time of secretion is not restricted to the period of budding, that the site of secretion is not restricted to a special region of the zooid, and that a bud is attracted not only by its mother but also by any other zooid in the same colony.

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